Patent application of

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For

Methods for the treatment or prophylaxis of disease by inhibition of ornithine decarboxylase.

Field of the Invention

The present invention relates to methods of treatment or prevention of disease using salts of 2-difluoromethyl-2,5-diaminopentanoic acid (DFMO) with chitosan.

Background-Cross-References to Related Application

This is a divisional of United States Patent Application Serial Number: 09/919692 filed on 07/31/2001, the entire disclosure and contents of which are incorporated by reference.

Technical field:

This patent relates to the therapeutic use of salts of 2-difluoromethyl-2,5-diaminopentanoic acid (DFMO) with chitosan. DFMO, in vitro and in vivo, is an inhibitor of ornithine decarboxylase, an enzyme that is involved in polyamine formation in organisms.

Background of the invention:

In both eukaryotic and prokaryotic cells, the decarboxylation of ornithine to putrescine, a reaction catalyzed by ornithine decarboxylase (ODC), is the first step in the biosynthesis of the polyamines known as spermidine and spermine. The polyamines, which are found in animal

tissues and microorganisms, are known to play an important role in cell growth and proliferation. The onset of cell growth and proliferation is associated with a marked increase in ODC activity and an increase in the levels of putrescine and the polyamines. Although the exact mechanism of the role of the polyamines in cell growth and proliferation is not known, it appears that the polyamines may facilitate macromolecular processes such as DNA, RNA, or protein synthesis. (Tabor H, Tabor CW, Cohn MS, Hafner EW. Streptomycin resistance produces an absolute requirement for polyamines for growth on an Escherichia coli strain unable to synthesize spermidine. J Bacteriolol 1981; 147: 702-4; Mamont PS, Bohelen, P, McCann PP, Bey P, Schuber R, Tardif C. Alpha-methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture. Proc Natl Acad Sci USA 1976; 73: 1626-30.)

The association between high levels of the polyamines and rapid proliferation was discovered more than a quarter of a century ago. (Bachrach U and Weinstein A. Effect of aliphatic polyamines on growth and macromolecular syntheses in bacteria. J. Gen. Microbiol., 60: 159-165 1970.) Subsequent studies showed that activation of the enzyme ODC was important for carcinogenesis and subsequent tumor development in animal and tumor models. (Weeks CE, Harmann AL, Nelson FR, Slaga TJ. Alpha difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits tumor promoter-induced polyamine accumulation and carcinogenesis in mouse skin. Proc Natl Acad Sci USA 1982; 79:6028-32.)

It is currently known that increased intracellular polyamine concentrations are related to human neoplastic conditions. (Verma, AK Inhibition of tumor promotion by DL-alpha-difluoromethylornithine, specific irreversible inhibitor of ornithine decarboxylase. Basic Life Sci., 52:195-204, 1990). A further example of this relationship between high polyamine concentrations and neoplasms involves colonic polyps and cancers compared to surrounding normal colon mucosa. (Hixson, LJ, Garewal, HS, McGee D., Sloan D, Fennerty, MB, Sampliner RE and Gerner EW. Ornithine decarboxylase and polyamines in colorectal neoplasia and adjacent mucosa. Cancer Epidemiol. Biomark. Prev. 2;369-374, 1993; Rozhin J, Wilson PS, Bull AW, and Nigro, ND.

Ornithine decarboxylase activity in the rat and human colon. Cancer Res. 44: 3226-3230, 1984.)

Other groups have reported that polyamine metabolism was necessary for carcinogenesis, especially in epithelial tissues. ODC inhibitors have been found to inhibit or suppress tumor formation in models of bladder, breast, colon and skin carcinogenesis. (Verma, AK Inhibition of tumor promotion by DL-alpha-difluoromethylornithine, specific irreversible inhibitor of ornithine decarboxylase. Basic Life Sci., 52:195-204, 1990; Nigro ND, Bull AW and Boyd, ME. Inhibition of intestinal carcinogenesis in rats: effect of difluoromethylornithine for colon cancer prevention. J. Natl Cancer Inst. 77: 1309-1313, 1986; Thompson HJ, and Ronan Am. Effect of DL-2-difluoromethylornithine and endocrine manipulation on the induction of mammary carcinogenesis by 1-methyl-1-nitrosourea. Carcinogenesis (Lond.), 7: 20032006, 1986.)

It is thought, however, that the mechanism of cancer prevention by ODC inhibitors such as DFMO may involve more than just inhibition of cell proliferation. Animal studies show that DFMO may act at later stages in models of chemical carcinogenesis. These stages involve the transition of non-invasive tumors to invasive cancers. (Slaga, TJ. Multistage skin carcinogenesis: a useful model for the study of the chemoprevention of cancer. Acta Pharmacol. Toxicol., 55 (Suppl. 2): 107-124, 1984.)

DFMO has been studied and continues to be studied as a cancer prevention agent, especially in skin, cervical and colon cancer. (Love RR, Carbone, PP Verma, AK, Gilmore D, Carey P, Tutsch KD. Pomplun M, and Wilding G. Randomized Phase I chemoprevention dose-seeking study of alpha-difluoromethylornithine. J. Natl. Cancer Inst., 85:732-736, 1993; Nishioka K, Melgarejo AB, Lyon RR and Mitchell MF. Polyamines as biomarkers of cervical intraepithelial neoplasia. J. Cell. Biochem., 23 (Suppl.): 87-95, 1995; Mitchell MF, Tortolero-Luna G, Lee JJ, Hittelman WN, Lotan R, Wharton JT, Hong, WK and Nishioka, K. Phase I dose de-escalation trial of alpha-difluoromethylornithine in patients with grade 3 cervical intraepithelial neoplasia. Clin. Cancer Res.., 4:303-310, 1998; Meyskens Fl, Emerson SS, Pelot D, Meshkinpour H, Shassetz R, Einspahr J, Alberts DS, and Gerner, EW. Dose de-escalation chemoprevention trial of alpha-

difluoromethylornithine in patients with colon polyps. J. Natl. Cancer Inst., 86:1122-1130, 1994.)

While high doses of DFMO in humans can cause some problems with hearing (reversible upon discontinuation of DFMO), at the doses used for chemoprevention of cancer (0.50 g/m2/day) such concerns have been found to be groundless. (Meyskens FL, Gerner E, Emerson S, Pelot D, Durbin T, Doyle K and Lagerber W. A randomized double-blind placebo controlled Phase IIb trial of difluoromethylornithine for colon cancer prevention. J. Natl. Cancer Inst., 90: 1212-1218, 1998).

DFMO has also been found useful in conditions unrelated to cancer. ODC inhibitors have been associated with control of hair growth. Studies in mice have suggested that the ODC gene is an important regulatory gene for the mouse hair follicle. (Soler AP, Gilliard G, Megosh LC, O'Brien TG.J Modulation of murine hair follicle function by alterations in ornithine decarboxylase activity. Invest Dermatol 1996 May;106(5):1108-13.) The FDA, to control facial hair growth in women, has recently approved DFMO. (Current DFMO salts, when used topically, cause burning, irritation and inflammation.) DFMO may have use in controlling male facial hair growth as well and may constitute a methodology to supplant or reduce the use of razors to remove facial hair in men.

Review of Prior Art

United States Patent 4,330,559, May 18, 1982, Bey, et al. discloses the use of DFMO to treat benign prostatic hypertrophy. United States Patent 4,399,151, August 16, 1983, Sjoerdsma, et al. discloses the use of 2-(difluoromethyl)-2,5-diaminopentanoic acid (DFMO) for inhibiting the growth of protozoa. United States Patent 4,405,530, September 20, 1983, Gerhart, discloses the preparation of fluorinated amino-nitriles. These patents do not disclose the use of salts of DFMO with chitosan.

United States Patent 4,413,141, November 1, 1983 Bey, et al. discloses 2-(difluoromethyl)-2,5-

diaminopentanoic acid (DFMO) and the methods for the preparation and use thereof. United States Patent 4,499,072, February 12, 1985, Sunkara, et al. discloses the use of DFMO as an ODC inhibitor along with interferon in treating diseases. United States Patent 4,720,489, January 19, 1988, Shander, discloses the use of DFMO as an ornithine decarboxylase inhibitor to modify hair growth. These patents do not disclose the use of salts of DFMO with chitosan.

United States Patent 5,648,394 July 15, 1997, Boxall, et al. discloses the use of DFMO as a topical composition for inhibiting hair growth but does not teach the use salts of DFMO with chitosan. WO9814188, 1998-04-09, Love et al. teaches the use of preparations comprising a single enantiomer or defined ratio of enantiomers of alphadifluoromethylornithine (DFMO) for treating, preventing, controlling the growth of and/or reducing the risk of developing estrogen independent breast cancer or tumor and for administering DFMO alone or in combination with taxol. However, this patent does not teach the use of salts of DFMO with chitosan. United States Patent 5,851,537, Dec. 22, 1998, Alberts et al. discloses the use of topical application of DFMO to prevent skin cancer but does not teach the salts of DFMO with chitosan. WO0069434, 2000-11-23, Love discloses the use of Celecoxib, a COX-2 specific nonsteroidal antiinflammatory agent, in combination with DFMO for the prevention and/or treatment of cancers. However, this patent does not teach the salts of DFMO with chitosan. United States Patent 6,166,079, December 26, 2000, Follen et al. discloses the use of DFMO for the treatment or prevention of cervical intraepithelial neoplasia. United States Patent 6,258,845, July 10, 2001, Gerner, et al. discloses the use of DFMO and sulindac combination in cancer chemoprevention. These patents do not teach the use of salts of DFMO with chitosan.

Administration of agents that inhibit ornithine decarboxylase would have significant utility over a wide range of disorders or conditions associated with an increase polyamine metabolism. For example, in addition to the prevention and/or treatment of different types of cancer or precancer conditions, such agents would have utility in preventing and/or treating colon polyps, benign prostatic hypertrophy (BPH), or hirsutism. Such agents may also provide a means to decrease the need for daily shaving of facial hair in males.

Accordingly, there is a need in the art for methods related to the use of such ODC inhibition agents to prevent and/or treat conditions associated with increased polyamine metabolism. The present invention fulfills this need, and provides further related advantages.

Summary of the invention:

Briefly stated, the present invention discloses methods for the use of salts of DFMO with chitosan. These salts of DFMO with chitosan have utility in treating or preventing a variety of conditions related to the aforementioned mechanisms of action of DFMO, namely ODC inhibition. Thus in one embodiment, a salt of DFMO with chitosan is administered to a warmblooded animal in need thereof to inhibit ODC. In yet a further embodiment, a salt of DFMO with chitosan is administered to a warm blooded animal to prevent and or treat the following conditions: aging of the skin, cancer, HIV, alopecia, solar keratosis, benign prostatic hypertrophy, prostate cancer, breast cancer, cervical cancer, and other such conditions in which polyamine metabolism requires modulation. Such a salt may be administered along with any other agent to enhance its therapeutic effectiveness. Other aspects of the present invention will become evident upon reference to the following detailed description.

Detailed description of the invention:

As mentioned above, this invention is generally directed to therapeutic uses of salts of DFMO with chitosan. Such salts of DFMO with chitosan, when administered to a warm-blooded animal in need thereof, have utility in the prevention or treatment of conditions enumerated above in warm-blooded animals, including humans.

The term "treat" or "treatment" means that the symptoms associated with one or more conditions mentioned above are alleviated or reduced in severity or frequency and the term "prevent" means that subsequent occurrences of such symptoms are avoided or that the frequency between such occurrences is prolonged.

It has now surprisingly been found that salts of DFMO with chitosan have good characteristics that are such as to render them particularly suitable both for use in pharmaceutical formulations and for preparative applications.

The example illustrates the complete absence of the well known irritation side effects of DFMO when a cream containing 20% salt of DFMO with chitosan is applied topically to the forearm of healthy volunteers.

This example is given to illustrate the present invention, but not by way of limitation. Accordingly, the scope of this invention should be determined not by the embodiments illustrated, but rather by the appended claims and their legal equivalents.

Example 1:

A cream containing 20% salt of DFMO with chitosan was applied to the forearm of 10 healthy individuals twice daily for a two-week period in an outpatient clinic. No patients complained of burning, irritation, scaling or redness after the cream. Patients returned to the clinic after having used the cream for two weeks for a visual inspection of the forearm area. The examining physician noted no redness, irritation or scaling in the area where the cream had been applied.